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Novel Transcribed Sequences Neighbouring a Translocation Breakpoint Associated With Schizophrenia

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A 1.3Mb chromosome 11-specific yeast artificial chromosome (YAC) that spans a t(1;11) translocation breakpoint associated with major psychosis has been used to enrich cDNAs that are encoded within it and expressed in the human foetal brain. Database analysis of the selected fragments led to the identification of 54 clones matching α-tubulin, 4 fragments matching two anonymous human expressed sequence tags (ESTs) and 8 fragments giving no database matches. The clones matching α-tubulin led to the identification of a novel α-tubulin locus located approximately 250 kb proximal to the translocation breakpoint. Extensive sequence and expression analysis of this locus suggests that this is a processed pseudogene, although a long open reading frame is maintained and the possibility that an abnormally acting protein may be expressed in a highly tissue or developmental specific manner cannot be discounted. The novel cDNA fragments map up to 700 kb proximal to the translocation breakpoint and are associated with potential CpG islands. Reverse transcriptase polymerase chain reaction (RT-PCR) expression analysis and high resolution genomic mapping suggest that they may comprise up to three novel genes. No major disruption of the identified fragments could be detected in the genomic DNA of translocation carriers. The psychosis associated with this translocation may therefore be due to position effects on the transcription of these genes or an involvement of translocated chromosome 1 sequences. *Am. J. Med. Genet. 74:82–90, 1997.*

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INTRODUCTION

Family, twin and adoption studies have provided conclusive evidence that schizophrenia and the related psychoses are, at least in part, genetic disorders [Prescott and Gottesman, 1993]. Attempts to define the underlying genes by linkage and candidate gene approaches have, however, been plagued by factors such as variable diagnostic boundaries, probable aetiologic heterogeneity and polygenic non-Mendelian inheritance patterns [Cloninger, 1994]. Thus far, no robust and undisputed associations between schizophrenia and particular candidate genes have been found. Recent worldwide collaboration and improvements in linkage methodology have enabled the detection of various candidate regions, for instance on chromosomes 6p [Wang et al, 1995] and 22q [Pulver et al., 1994; Coon et al., 1994], but further replication and refinement is required.

An alternative approach to schizophrenia gene identification involves screening for cytogenetic abnormalities which cosegregate with psychotic illness, thus defining a starting point for linkage and positional cloning endeavours. One such abnormality was found in a large Scottish pedigree, in which major mental illness is tightly linked to a balanced translocation t(1;11)(q42.1;q14.3) [St. Clair et al., 1990]. A maximum LOD score of 4.34 is generated with a broad definition of affected status (schizophrenia, schizoaffective disorder, major depression, adolescent conduct and emotional disorder). This strongly suggests that a gene or genes involved in major mental illness in this family reside at or near this translocation breakpoint on chromosomes 1 and/or 11. The translocation event may exert either a direct effect on the coding sequence of this gene(s) or the control of its transcription. Alternatively, the translocation could merely represent a marker for a mutation in a cosegregating gene.

Fine mapping and cloning of the region surrounding the chromosome 11 breakpoint region has been de-

scribed previously [Fletcher et al., 1993; Muir et al., 1995; Evans et al., 1995]. These efforts identified a yeast artificial chromosome (YAC), ICRF y901 D0485, which spans the translocation breakpoint on chromosome 11. This large YAC (1.3 Mb) provides a useful resource from which to begin a search for genes at and around the chromosome 11 breakpoint. Here we describe the isolation of transcribed sequences from this YAC using an optimised method for "direct cDNA selection" [Lovett et al., 1991; Parimoo et al., 1991; Brookes et al., 1995]. Whilst no genes were found that directly spanned the chromosome 11 translocation breakpoint, we did identify novel transcribed elements, some associated with a potential CpG island, up to 700 kb proximal to the breakpoint, as well as an α-tubulin related sequence 250 kb proximal to the breakpoint.

MATERIALS AND METHODS

cDNA Enrichment

Human foetal brain cDNA was prepared as described elsewhere [Brookes et al., 1995]. ICRF yacD0485 (obtained from H. Lehrach, ICRF Reference Library Database, Berlin, Germany) was purified from yeast chromosomes by preparative pulsed field gel electrophoresis in 1% low melting point agarose without exposure to ethidium bromide or UV light [as described in Maule et al., 1994]. The DNA was recovered by agarase (Boehringer Mannheim, Germany) treatment and ethanol precipitation, cDNA enrichment was performed by reacting the above DNAs together as described elsewhere [Brookes et al., 1995] additionally including 2 μg of sonicated yeast genomic DNA plus 2 μg of sonicated human ribosomal DNA clones pA and pB (obtained from Dr. I. Gonzalis, Hahnemann University, Philadelphia, PA) for pre-annealing of both resources. Product cDNA fragments were finally cloned into pBluescribe (pBS); (Stratagene, La Jolla, California) via *EcoRI* sites engineered into the linkers.

Polymerase Chain Reactions

All PCRs entailed 30 cycles on a Hybaid Omnigene employing tube temperature control. Reactions employed 1 unit Amplitaq (Perkin Elmer, Norwalk, Connecticut), 600 ng of a single primer or 300 ng each of two primers, 200 μM of each dNTP, and were conducted in 50 μl reaction buffer (10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.1% Triton X-100). PCR products were examined on 0.8–1.5% agarose gels stained with 250 μg/ml ethidium bromide. Table I details the primers and reaction conditions for each PCR.

Probes and Hybridisations

Plasmid clone inserts were PCR-amplified from 96°C heat-lysed bacterial suspensions [Taylor, 1991] using primers 291 and 292 (see Table 1). PCR products were digested with *EcoRI* to remove the vector arms, and the insert isolated by low melting point agarose gel purification. Gel slices were labelled directly by α^{32}P-dCTP random priming [Feinberg and Vogelstein, 1984]. Southern blots and bacterial colony lifts were prepared according to standard procedures [Sambrook et al., 1989]. Filters were prehybridised at 68°C for 2 hours in 5 × SSC, 10% dextran sulphate, 0.1% sodium pyrophosphate, 0.1% sodium dodecyl sulphate, 0.1% ficoll, 0.1% polyvinylpyrolidone, 0.1% bovine serum albumin and 100 μg/ml denatured sonicated salmon sperm DNA. Probes containing repeat elements were preannealed with 250 μg unlabelled sonicated total human genomic DNA at 68°C for 30 min. Probes were then added to the prehybridisation and incubation continued at 68°C overnight. Filters were washed in 0.1 × standard saline solution (SSC), 0.1% sodium dodecyl sulphate at 68°C and exposed to Kodak X-OMAT X-ray film or to a Molecular Dynamics phosphor-imager screen.

Sequencing and Sequence Analysis

Plasmid inserts were examined by fluorescence cycle sequencing on an Applied Biosystems 373A DNA sequencer according to the manufacturer's instructions. PCR products were sequenced by a radioactive dideoxy terminator sequencing method adapted from Winship, [1989]. After agarose gel purification, agarase treatment, phenol:chloroform extraction and ethanol precipitation, PCR products were denatured by heating to 90°C for 3 minutes in the presence of 300 ng sequencing primer, 10% dimethylsulphoxide and 1x reaction buffer from the Sequenase Version 2.0 kit (USB, Cleveland, Ohio), and snap-frozen on dry ice. Annealing and termination steps were performed using the above sequencing kit according to the manufacturer's instructions, with the addition of 10% dimethylsulphoxide to each termination mix. Sequencing ladders were resolved on 6% denaturing polyacrylamide gels and the bands visualised by exposure to Kodak X-OMAT X-ray film. Derived sequences were used to search GenBank, Swissprot and dbEST databases at the protein and DNA level using the BLAST [Altschul and Gish, 1990] and Fasta [Pearson and Lipman, 1988] programs.

Extraction of Total RNA and Preparation of cDNA

Total RNA was extracted from a variety of human tissues using RNazol B™ (Biogenesis Ltd., Poole, UK) according to the manufacturer's instructions. The RNA was treated with 10 u/μg DNase I (Boehringer Mannheim) in the presence of 10 u/μg RNase Inhibitor (Boehringer Mannheim), extracted with phenol:chloroform and ethanol precipitated. The resulting pellet was resuspended in diethyl pyrocarbonate (DEPC)-treated water. First strand cDNA synthesis was performed using the random hexamer primer from the First Strand cDNA Synthesis kit (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions. Two to five μl of the resulting cDNA was then used directly in a standard PCR reaction.

RESULTS

Human foetal brain cDNAs represented in the translocation breakpoint spanning YAC, ICRF y901 D0485, were physically enriched by a variation of the method of Brookes et al. [1995], as described in Materials and Methods. After plasmid cloning of the recovered cDNA fragments, a set of 73 random recombinants were chosen and their inserts sequenced. This revealed 3 products containing high copy repeat elements (two of

TABLE I. PCR Primers and Conditions*

PCR reaction (Conditions/annealing temp.)	Primers
Amplification of cDNA selection product (NH, 54°C)	477: 5′ CCG AAT TCT AGA GTC GAC C 3′
Amplification of plasmid clone inserts (NH, 52°C)	291: 5′ CAG GAA ACA GCT ATG AC 3′ 292: 5′ GTA AAA CGA CGG CCA GT 3′
α-tubulin locus-specific assay (TD 65–55°C)	711: 5′ CTG TCA CAG GTG GGA GGG GAT TAA AGA GAT ATA AAC AAC TA 3′ 712: 5′ GTC GAC TTT CAA TTT TCA AGT CTC ATT TGT TCA GCA GTA CAT TTC A 3′
α-tubulin RT-PCR (TD 66–56°C)	496: 5′ GGG AGG AGA TGA CTC CTT CAA CAC CTT CAG 3′ 497: 5′ ATG GCT GTG GTG TTG CTC AGC ATG CAC TCT 3′
Genomic PCRs:	
c1–c2 (L, TD 68–58°C)	485: 5′ ATT CGG TGA TGC CTC TGT GTC TGT C 3′ 487: 5′ CAC AGA TSC AAA AGC CCC AGC CAG T 3′
c3–c4 (TD 70–60°C)	488: 5′ TCC AAA TGG CAT CTC CTA CCC TAT C 3′ 491: 5′ GCG GGG CAG GAG TGT GTA CTT CTT C 3′
c3–c5 (L, TD 68–58°C)	489: 5′ GCA AYG CAA GGA CAT CTA ATA CAG C 3′ 493: 5′ GTC ACT GTC CAC GGC AGC CCA GCA T 3′
c3–c6 (TD, 70–60°C)	489: as above 495: 5′ TCC ACA CAT ATC TTC TAT CAT CCC A 3′
c4–c5 (L, TD 68–58°C)	491: as above 493: as above
c4–c6 (L, TD 68–58°C)	491: as above 495: as above
RT-PCRs:	
c3–c6 (TD 60–50°C)	489: as above 495: as above
C2 (TD 70–60°C)	486: 5′ CAT ATC TCA CAT CCT CAG AAA GGC T 3′ 487: as above
cDNA clone 207275 (C1) (TD 65–55°C)	840: 5′ AGT AGT TGG TGA TGA TAG CG 3′ 841: 3′ CAC CGT TGC CTT GGA CAC TG 3′

* Long range PCR was carried out where indicated (L) using an "Expand" long PCR kit (Boehringer Mannheim) according to the manufacturer's instructions. A Hot Start (90°C for 5 min) [Chou et al. 1992] was used unless otherwise stated (NH). Denaturation was performed at 93°C or 94°C, for 30 sec in the first cycle and 15 sec thereafter. All annealing steps were for 30 sec. All extension steps were performed at 72°C; the length of extension steps varied according to the length of the target product (approximately 1 minute per kb). "Touch down" PCR (TD) [Don et al., 1991] involved decreasing the annealing temperature by 2°C each cycle from the higher temperature until the lower temperature is reached. Subsequent annealing steps were performed at the lower annealing temperature.

which were "Alu" repeats) and 11 other distinct sequences represented by single clones or overlapping groups of clones. In order to check the validity of these 11 singlets/groups, a representative of each was checked for its ability to hybridise back to the input DNAs. Four singlets failed to hybridise to the YAC DNA and were therefore deemed to be artefactual and excluded from further analysis. The remaining 7 gave signals when tested against both the YAC DNA and the foetal brain cDNA. Database searching with the 7 product sets (66 clones) identified 1 set (54 clones) related to an α-tubulin gene, 1 set (4 clones) highly related to two anonymous human expressed sequence tags (ESTs); (accession numbers H59660 and N33739), and 5 sets (1 set of 4 clones and 4 singlets) with no significant database matches.

An α-Tubulin-Related Locus

Three distinct α-tubulin gene sequences exist in current databases, "foetal brain α-tubulin," "keratinocyte α-tubulin" and "testis specific α-tubulin," which are different in their untranslated regions but virtually identical in their coding domains [Cowan et al., 1983] (Fig. 1). Testis-specific α-tubulin has been mapped to chromosome 2q [Gerhard et al., 1985] but the map position of the other two subtypes is unknown. The cDNA fragments that we isolated cover 90% of the 1.35 kb α-tubulin coding sequence with 8 clones extending into untranslated regions. Of these, 3 were likely to have been derived from the foetal brain-specific α-tublin gene, 3 from the keratinocyte α-tubulin gene, and 2 showed novel untranslated regions indicating a previously unrecognised α-tubulin subtype. As expected, no cDNAs were derived from the testis-specific α-tubulin since only foetal brain cDNA was used for cDNA selection.

Representative α-tubulin products were probed onto long range restriction digests of YAC D0485. This revealed a single α-tubulin-related locus approximately 250 kb centromeric to the translocation breakpoint, a

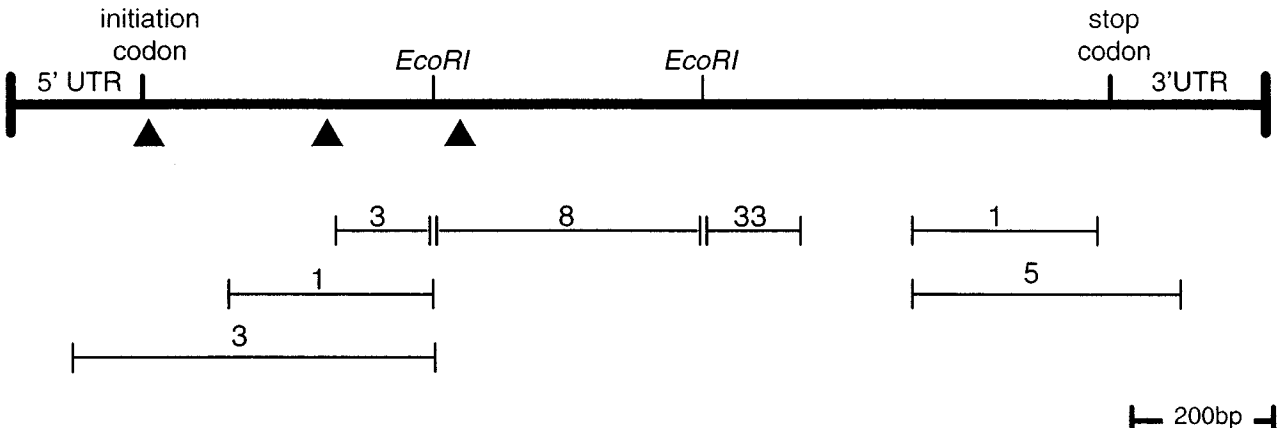

Fig. 1. Position and frequency of recovered α-tubulin cDNA fragments (thin black lines) relative to the foetal brain and keratinocyte α-tubulin cDNAs (thick black line). The positions of start and stop codons and *EcoRI* sites are marked. Positions of introns in foetal brain α-tubulin are shown by triangles. Of clones which extend into the 5′ untranslated region, 1 matches keratinocyte α-tubulin and 2 are from a novel family member. Of clones which extend into the 3′ untranslated region, 3 match foetal brain α-tubulin and 2 match keratinocyte α-tubulin.

position not associated with any rare cutter restriction enzyme sites indicative of CpG islands (see Fig. 2) [Maule et al., manuscript in preparation]. This locus presumably explains the enrichment of the α-tubulin cDNAs, and may be equivalent to a previously reported α-tubulin locus on chromosome 11 that was not precisely mapped [Gatti et al., 1987].

To examine further the α-tubulin-related locus in D0485, we subcloned partial *Sau3AI* digest fragments from this YAC into a plasmid vector. Desired recombinants were selected by hybridisation with 5′ and/or 3′ α-tubulin probes and their inserts completely sequenced to at least threefold redundancy, mostly bidirectional. The D0485 α-tubulin-related locus was thus shown to be highly similar to keratinocyte α-tubulin, but it lacked introns. As summarised in Figure 3, most of the sequence differences in the D0485 α-tubulin-related locus relative to the keratinocyte gene are located in the untranslated regions, whilst coding sequence alterations are minor and do not disrupt the reading frame. The only exception to this is a 7 base pair deletion towards the 3′ end that imposes a premature termination codon a further three amino acids downstream and limits the potential open reading frame to the equivalent of the 5′ 80% of the keratinocyte α-tubulin gene.

The detailed structure of the YAC-encoded α-tubulin locus as represented in normal and translocation chromosomes was next examined. For this a 2.6 kb locus-specific PCR assay was devised employing primers (711 and 712) designed to match sequences immediately upstream and downstream of the YAC α-tubulin locus. This assay was applied to both normal genomic DNA and DNA from a somatic cell hybrid (MIS 7.4) [Fletcher et al., 1993] that contains a translocation-derived but not a normal chromosome 11. Control PCRs on water, mouse and hamster DNA were negative. Amplified products were sequenced producing unambiguous data for the entire α-tubulin locus plus 691 bp upstream and 619 bp downstream. No differences were seen between the sequence derived from the normal and the translocation chromosomes.

Loci for Novel cDNA Fragments

The 12 product cDNA fragments not related to α-tubulin comprised 2 contigs, C1 (4 clones, consensus of 406 bp) and C2 (4 clones, consensus of 230 bp), and 4 singlet clones, C3 (253 bp), C4 (218 bp), C5 (208 bp) and

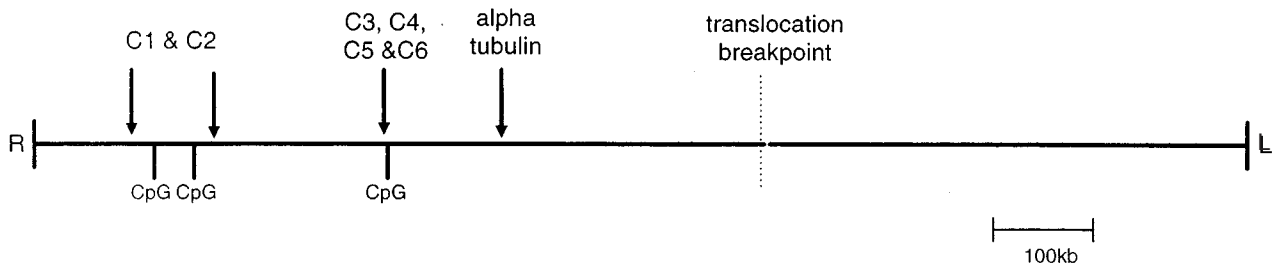

Fig. 2. Schematic diagram of the 1.3Mb YAC D0485 (L, left end; R, right end) showing approximate positions of the α-tubulin-related locus and the novel cDNA fragment contigs relative to potential CpG islands (defined by coincidental *Not I* and/or *BssHII* and *Eag I* sites) and the translocation breakpoint.

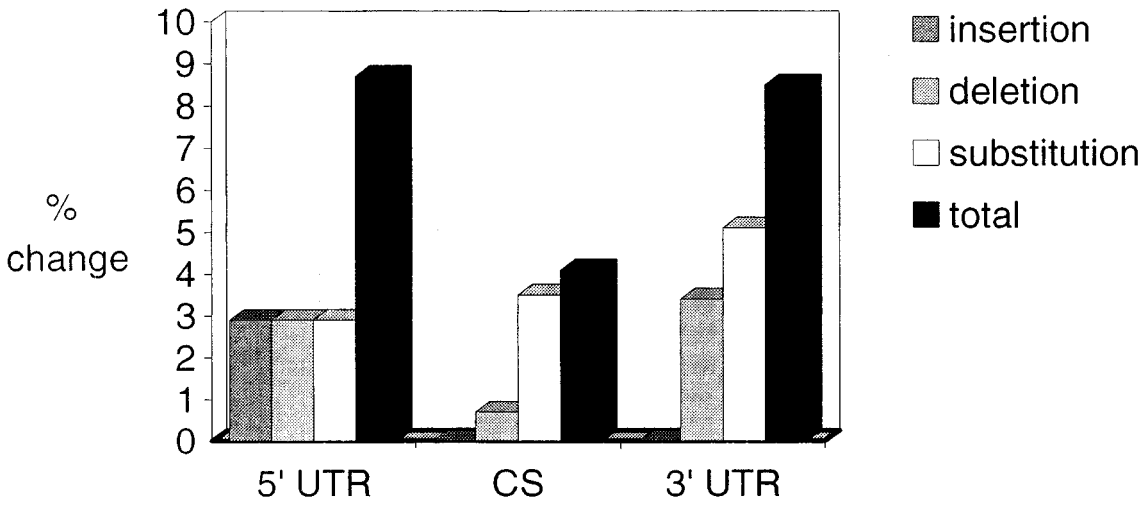

Fig. 3. Graph depicting the percentage of nucleotide insertions, deletions and substitutions in the D0485 α-tubulin locus relative to the keratinocyte α-tubulin gene. CS, coding sequence. UTR, untranslated region. Three of the 10 deleted bases in the coding sequence occur together, deleting phenylalanine at amino acid 52. The other 7 also occur together and change the reading frame. Of the single base substitutions in the coding sequence, 24 out of 47 (51%) alter the third base in the codon, only one resulting in a changed amino acid residue. Of the first or second base substitutions, 17 out of 23 (74%) result in a conservative change.

C6 (508 bp). Matches between C1 and two human dbEST cDNA sequences (98% identity in 102 bp overlap with EST N33739, 98.5% identity in 260 bp overlap with EST H59660) indicate this contig is part of a real gene.

The cDNA clone from which EST H59660 was derived (I.M.A.G.E. Consortium cDNA clone 207275) was obtained from Research Genetics Inc., Huntsville, Alabama, and its 1.2 kb insert was completely sequenced. The C1 sequence extended beyond the 5′ end of this clone, giving a total sequence of 1,393 bp, which extends to the polyadenylation signal and poly(A) tail at the 3′ end of the cDNA but is incomplete at the 5′ end. Database searches using this extended sequence yielded no further matches.

A clone from each contig was probed onto YAC D0485 DNA and total human genomic DNA, both digested with *EcoRI*. In the YAC DNA, C3, C4, C5 and C6 detected single bands, C1 hybridised to two bands and C2 to four bands. Bands of identical size were detected in the genomic DNA tracks. However, all clones, except C2, produced additional genomic bands: 1 for C3 and C6, at least 1 for C5 (other bands may have been masked by a smear caused by a repetitive element in the probe), 3 for C4 and 4 for C1. Probing *EcoRI* and *HindIII* digests of normal and translocation carrier genomic DNAs with these clones revealed no gross rearrangements in the YAC-hybridising sequences. When used to probe long range restriction digests of the YAC, all of the clones mapped towards one end of D0485, centromeric to both the translocation breakpoint and the α-tubulin-related locus (Fig. 2). Finer resolution restriction mapping showed that the loci corresponding to C3, C4, C5 and C6 lie tightly clustered within a 6.5kb *Sac I* fragment, which itself lies 7.7 kb from a potential CpG island (as defined by the presence of 1 *Not I* site, 1 *BssHII* site and 2 *Eag I* sites); (Maule et al., manuscript in preparation).

PCR primers were designed for both strands of each product clone/contig and used in all pairwise combinations for PCRs upon YAC D0485 DNA, with the aim of investigating the relative orientation of the products and the distance between them in genomic DNA. This defined two groups, C1/C2 (6.5 kb apart) and C3/C4/C5/C6 (total length of cluster 6.4 kb; see Fig. 4). The intra-clone/contig PCR products for C2 and C6 were larger than predicted by their cDNA sequences, by approximately 1.15 kb for C2 and 240 bp for C6. The ends of these PCR products were therefore sequenced, revealing splice junctions and introns internal to sequences matching the C2 and C6 cDNAs. While C2 showed consensus splice donor and acceptor sites, the splice acceptor consensus for C6 did not occur at exactly the position of reconvergence between genomic and cDNA sequence, probably implying that the C6 cDNA was encoded by a related gene elsewhere in the genome. The genomic and cDNA PCR products from C1 and C3–C5 showed essentially identical sequences.

Expression Studies

To test for possible expression of the D0485 α-tubulin-related locus, we devised an allele-specific assay for a 1,013 bp coding region fragment, employing primers (496 and 497) whose 3′ ends terminated at regions of difference between the D0485 and keratinocyte α-tubulin sequences. The efficiency and specificity of this primer combination was confirmed by application to total human genomic DNA and sequencing of the PCR product. The primers were then used for reverse transcription PCR (RT-PCR) upon a variety of DNase-treated, human total RNA samples (foetal brain, gestational age 10 weeks and 16 weeks, spleen, liver, limb, heart and kidney, and adult brain, colon, liver, prostate and testis). We also tested RNA from an immortalised

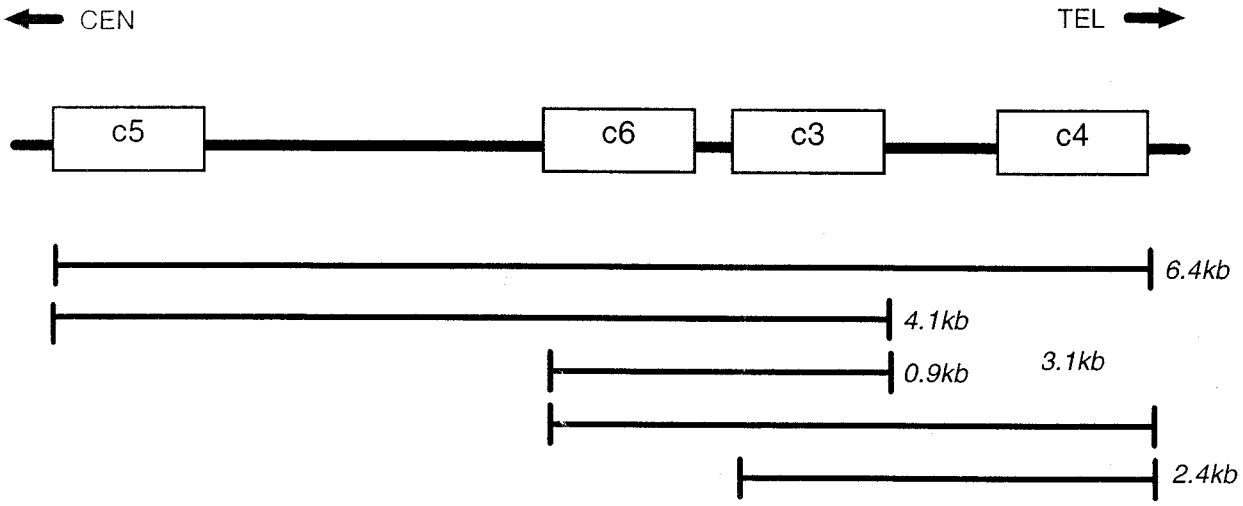

Fig. 4. Genomic organisation (genomic DNA, thick black line) of the YAC D0485 loci (boxes) corresponding to products C3–C6, as defined by PCR analysis. Interfragment PCR product lengths are given underneath. CEN = centromere, TEL = telomere.

lymphoblastoid cell line from a translocation carrier, as well as RNA from somatic cell hybrid MIS7.4 (containing the derived chromosome 11) [Fletcher et al., 1993]. No amplified DNA was produced by any of these samples. A positive control RT-PCR (a 422 bp portion of the glutaraldehyde phosphate dehydrogenase gene) gave the expected single band from all the above RNA samples.

RT-PCR was also used to test for expression of C1, C2 and C3/6, using the primers devised for PCR upon genomic DNA. RT-PCR between C3 and C6 produced a 748 bp fragment, which when sequenced revealed that these two fragments lie immediately adjacent to each other in mRNA. When RNA from the same tissue range as above was assayed (under nonquantitative conditions), C3/C6 was shown to be widely expressed with the highest levels detected in foetal brain of gestational age 10 weeks, and negligible expression in foetal kidney (Fig. 5c). RT-PCR of a 153 bp C2-derived product detected very high expression in adult testis, moderately high expression in foetal heart and a low level of expression in all other tissues (Fig. 5b). RT-PCR of a 435 bp fragment from cDNA clone 207275 (part of the same transcript as C1) revealed ubiquitous expression, with lower levels in adult brain and 10 week foetal brain (Fig. 5a). RT-PCR between products other than C3/C6 was attempted, but yielded no products.

DISCUSSION

We have searched for candidate schizophrenia genes in a chromosome 11-derived YAC (D0485) which spans a translocation breakpoint [Evans et al., 1995] linked to major psychosis in a large Scottish pedigree [St. Clair et al., 1990]. By physically enriching YAC-encoded foetal brain cDNAs, we identified a number of transcribed products. We utilised human foetal brain cDNA in this search due to the high proportion of genes expressed in the brain [Sutcliffe, 1988], and because of the likely tissue expression of a schizophrenia predisposing gene. The recovered cDNA fragments comprised α-tubulin sequences (including parts of a novel α-tubulin gene) plus 2 contigs and 4 singlet products representing up to three novel progenitor cDNAs or cDNA families.

The α-tubulin fragments were recovered due to a YAC sequence element with high similarity to the keratinocyte α-tubulin gene. This is located approximately 250 kb centromeric to the translocation breakpoint and not associated with a CpG island. Alpha tubulin is an attractive candidate gene for psychiatric disorders. Microtubules, which are comprised of α and β tubulin dimers, are involved in the maintenance and growth of axons and intracellular axonal transport [Cambray-Deakin and Burgoyne, 1987]. Tubulin also binds to several pre- and postsynaptic membrane components (for instance synapsin I and the $GABA_A$ receptor) and plays a role in signal transduction by binding to GTP and G-protein [Aubert-Foucher et al., 1990; Item and Sieghart, 1994; Roychowdbury and Rasenick, 1994]. Microtubule-associated proteins (MAPs), which bind to tubulin, have previously been implicated in the aetiology of the cytoarchitectural abnormalities characteristic of schizophrenia [Kerwin, 1993] and aberrant expression of MAP2 and MAP5 has been detected in the hippocampus of some schizophrenics [Arnold et al., 1991].

The D0485 α-tubulin-related sequence described here may be aberrantly expressed or repressed in individuals bearing the translocation, and therefore it may be involved in the aetiology of the psychiatric disorders in this family. If expressed, the resultant protein would be 80% of the normal length, and should still be able to form dimers, bind GTP, G-protein and the ATPase translocators [Jayaram and Haley, 1994; Rodionov et al., 1990], though its ability to form microtubules and bind to MAPs may be disrupted [Arévalo et al., 1990; Paschal et al., 1989]. Such a protein may exert a dominant negative effect via its interaction with nor-

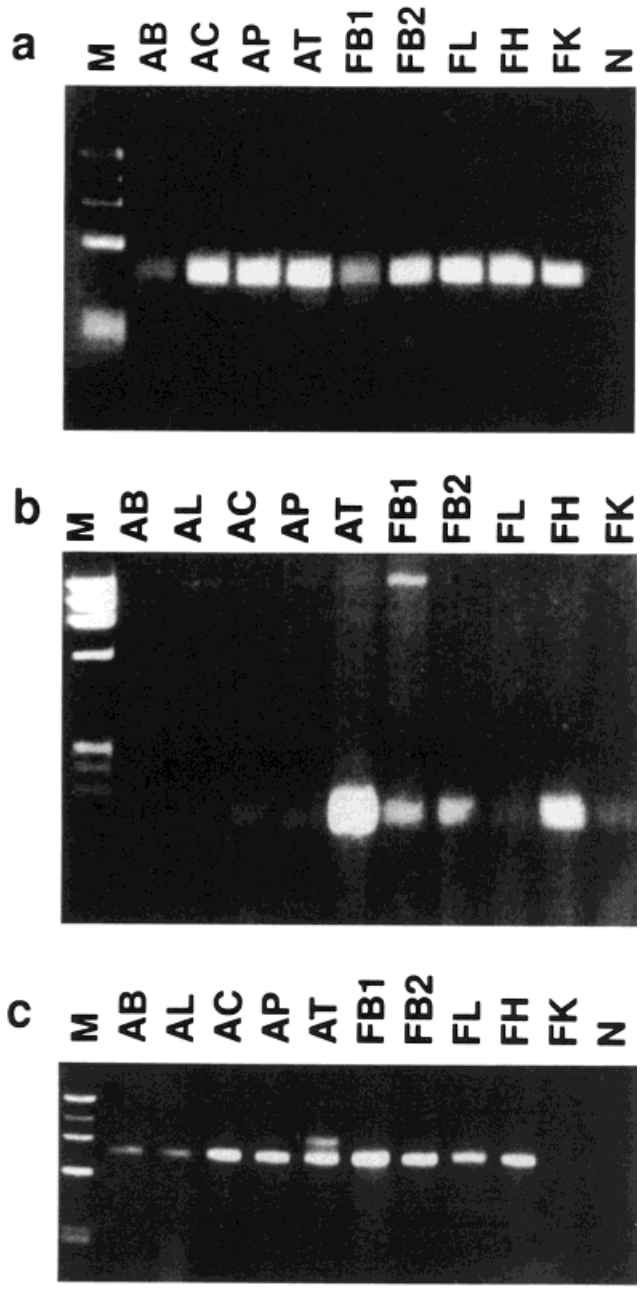

Fig. 5. RT-PCR expression analysis of (**a**) C1 (**b**) C2 and (**c**) C3 to C6. M, ϕx174 *Hae III* marker; AB, adult brain; AC, adult colon; AL, adult liver; AP, adult prostate; AT, adult testis; FB1, foetal brain (10 weeks); FB2, foetal brain (16 weeks), FL, foetal liver (13 weeks); FH, foetal heart (13 weeks); FK, foetal kidney (13 weeks); N, negative control (water as template). -RT controls performed for C1 on each tissue were negative (data not shown). A -RT control is not required for C2 and C3–C6 since the expected product size from DNA is different from that expected from RNA. The large (1.3 kb) band produced for C2 from 10-week foetal brain (b, track 7) and the upper band produced for C3–C6 from testis (c, track 6) resulted from incomplete DNase I digestion of the RNA sample, since their size is that expected from template genomic DNA, and they were not present in other RT-PCR reactions performed on RNA samples from the same tissue.

mal β-tubulin. The truncation of the coding sequence, the absence of introns in genomic DNA and the lack of detectable expression from a wide range of tissues suggests that this particular α-tubulin locus may be a processed pseudogene. However, it does retain a long open reading frame, and shows less divergence from the keratinocyte α-tubulin gene in its coding region relative to its untranslated regions. Hence, this element may be a functional gene that is expressed in a highly tissue or developmental stage-specific manner.

Both cDNA contigs C1 and C2 were shown to be derived from transcribed genes by their amplification from RNA by RT-PCR, also for C1 since it overlaps with two anonymous dbEST cDNAs, and for C2 since splicing differences were demonstrated between the YAC D0485 and the cDNA sequence. Since the cDNA sequences have not been extended to full length, it not yet known whether these contigs are derived from the same mRNA. It is most likely, however, that they are derived from separate genes, since they exhibited a different RT-PCR profile of expression from various tissues, and RT-PCR between them did not yield a product.

Within YAC D0485, the C1 and C2 contigs hybridise to 2 and 4 *EcoRI* fragments respectively, approximately 700 kb centromeric to the schizophrenia-associated translocation breakpoint, and in association with potential CpG islands. A long range restriction map of the region suggests that the multiple loci may be related to the presence of a long inverted repeat, although the exact details of the loci have not been determined (Maule et al., manuscript in preparation). Genomic Southern blot hybridisations demonstrate further C1 loci outside YAC D0485; hence, it is unclear whether the YAC D0485 C1 locus is transcriptionally active. Without unambiguous sequence data for all C1 and related cDNAs and their genomic counterparts, it is not possible to determine which may be functional or pseudogene copies. For C2 on the other hand, there are no genomic copies of the sequence outwith YAC D0485; therefore, at least one of the YAC copies is a functional gene.

The remaining products, C3–C6, all detect single loci in D0485 that are tightly clustered within 6.4 kb close to a potential CpG island. Products C3 and C6 were shown by RT-PCR to be adjacent in mRNA. Although C3/C6 could not be linked to either C4 or C5 using conventional RT-PCR methodology, their close proximity in genomic DNA strongly suggests that C3–C6 are parts of a single transcriptional unit which resides in YAC D0485 between the C1/C2 and α-tubulin loci. The loci do not form an uninterrupted open reading frame if joined, yet this could be due to errors in single pass sequencing and the presence of as yet undetected exons. Although genomic Southern blot hybridisations indicate additional C3–C6 copies elsewhere in the genome, a splice difference between the YAC D0485 and cDNA copies of C6 suggests that this copy is transcriptionally active. The other copies may correspond to other members of a gene family or pseudogenes. The polarity of the splice sites in C6 is consistent with the orientation of this locus as surmised from the position of the potential CpG island at the 5′ end of the gene.

All these YAC encoded elements are now schizophrenia candidate genes whose expression may be disrupted or induced by the nearby translocation. We did not detect any nucleotide changes in the YAC α-tubulin locus or gross mutations in C1, C2 or C3–C6 when we examined them in a cell line derived from a translocation carrier. Since full length cDNA sequence is not yet available for the novel genes, and nothing can be surmised about their function from database matches, it is impossible to theorise over how likely they are to be involved in the aetiology of psychiatric disorders.

In conclusion, we have identified up to four candidate gene sequences close to a chromosome 11 translocation breakpoint associated with schizophrenia. The possibility that a long range position effect alters the transcription of these genes in individuals bearing the translocation can now be investigated. Alternatively a mutation may be detected which cosegregates with the translocation. No gene was identified by cDNA selection that spanned the breakpoint on chromosome 11. Efforts are now underway to study the chromosome 1 component of the translocation in similar detail, and to sequence extensive regions immediately flanking both chromosome breakpoints. At the culmination of these studies, the improved understanding of the molecular basis of schizophrenia in this particular family will hopefully yield valuable general insights into the nature of this disorder.

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